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# Egg buoyancy of flounder, *Platichthys flesus*, in the Baltic Sea—adaptation to salinity and implications for egg survival

Anders Nissling[a,*], Sofia Nyberg[a], Christoph Petereit[b]

[a] Ar Research Station, Department of Ecology and Genetics, Uppsala University, SE-621 67 Visby, Sweden
[b] GEOMAR Helmholtz Centre for Ocean Research Kiel, D-24105 Kiel, Germany

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ABSTRACT

Vertical distribution of eggs as determined by the egg buoyancy, i.e. the difference in specific gravity between the egg and the ambient water, have profound implications for the reproductive success and hence recruitment in fish. Here variability in egg specific gravity of flounder, *Platichthys flesus*, was studied along a salinity gradient and by comparing two reproductive strategies, spawning pelagic or demersal eggs. Egg characteristics of 209 egg batches (covering ICES subdivisions (SD) 22–29 in the brackish water Baltic Sea) was used to reveal the significance of egg diameter and egg dry weight for egg specific gravity (ESG), subpopulations, and egg survival probabilities of pelagic eggs following a major saline water inflow event. As an adaptation to salinity, ESG (at 7 °C) differed ($p < 0.001$) between areas; three subpopulations of flounder with pelagic eggs: $1.0152 \pm 0.0021$ (mean ± sd) g cm$^{-3}$ in SD 22, $1.0116 \pm 0.0013$ g cm$^{-3}$ in SD 24 and 25, and $1.0096 \pm 0.0007$ g cm$^{-3}$ in SD 26 and 28, contrasting to flounder with demersal eggs, $1.0161 \pm 0.0008$ g cm$^{-3}$. Egg diameter differed ($p < 0.001$) between subpopulations; from $1.08 \pm 0.06$ mm (SD 22) to $1.26 \pm 0.06$ mm (SD 26 and 28) for pelagic eggs and $1.02 \pm 0.04$ mm for demersal eggs, whereas egg dry weight was similar; $37.9 \pm 5.0$ μg (SD 22) and $37.2 \pm 3.9$ μg (SD 28) for pelagic, and $36.5 \pm 6.5$ μg for demersal eggs. Both egg diameter and egg dry weight were identified as explanatory variables, explaining 87% of the variation in ESG. ESG changed during ontogeny; a slight decrease initially but an increase prior to hatching. Egg survival probabilities judged by combining ESG and hydrographic data suggested higher egg survival in SD 25 (26 vs 100%) and SD 26 (32 vs 99%) but not in SD 28 (0 and 3%) after the inflow event, i.e. highly fluctuating habitat suitability. The results confirm the significance of ESG for egg survival and show that variability in ESG as and adaptation to salinity is determined mainly by water content manifested as differences in egg diameter; increase in diameter with decreasing salinity for pelagic eggs, and decreased diameter resulting in demersal eggs.



## 1. Introduction

To accomplish survival of early life stages, i.e. the reproductive success and thus recruitment to the adult stock (cf fitness), different spawning strategies and different tactics have evolved in teleosts (e.g. Wootton, 1990). This may be manifested as e.g. the production of demersal vs pelagic eggs, and as selection of spawning area, e.g. in coastal areas or offshore. In the present study the significance of variability in egg specific gravity (ESG) for the reproductive success of flounder (*Platichthys flesus*) was studied along a salinity gradient and by comparing two different reproductive strategies, spawning offshore with pelagic eggs (eggs which float freely in the water column) and in coastal areas with demersal eggs (eggs developing on the bottom), respectively, suggesting egg specific gravity as being a major selection process.

Spawning pelagic eggs is the most common strategy of marine fishes and is acquired by uptake of water during the terminal growth of oocytes in the ovary, oocyte hydration, just prior to ovulation (Fulton, 1898; Craik and Harvey, 1987; Cerdà et al., 2007). By incorporation of fluid with lower osmotic potential than the seawater environment, and thus compensating for the denser parts of the egg, the yolk and chorion, the overall egg specific gravity is lowered (Craik and Harvey, 1987). Both demersal and pelagic eggs undergo hydration, but the degree of water uptake, accompanied by a significant increase in oocyte volume, differ between pelagic and demersal eggs. Typically, pelagic eggs have high water content, 90–92%, compared to demersal eggs, 60–70%, and thus lower egg specific gravity that allows for buoyancy (Craik and Harvey, 1987).

* Corresponding author.
*E-mail address:* anders.nissling@ebc.uu.se (A. Nissling).

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Depending on species specific habitat preferences for egg development, egg specific gravity and hence the vertical distribution vary among species, and furthermore between populations within species, as adaptations to local environmental conditions. E.g. for the extensively studied cod, *Gadus morhua*, egg specific gravity vary from ca 1.022–1.026 g $cm^{-3}$ at marine conditions in the Atlantic to ca 1.009–1.014 g $cm^{-3}$ in the brackish water Baltic Sea (Table in Jung, 2012; Table in Petereit et al., 2014). This is due to differences in water content, ca 92% vs ca 97%, and accordingly in egg diameter, ca 1.2–1.4 mm vs ca 1.5–1.8 mm, for marine and brackish water cod eggs, respectively (Thorsen et al., 1996; Jung, 2012; Petereit et al., 2014). The higher uptake of water in brackish water cod eggs result in a stretching of the chorion, i.e. thinner chorion, and a change in the chorion (a considerable part of the total egg mass) to egg volume ratio influencing the ESG (Kjesbu et al., 1992). As egg buoyancy is determined mainly by salinity (Sundby and Kristiansen, 2015) marine fishes that have managed to adapt to the brackish conditions in the Baltic Sea, display different egg characteristics (water content and egg diameter) compared to their marine counterparts; for cod resulting in neutral egg buoyancy at 10–20 psu vs at 27–33 psu (e.g. Vallin and Nissling, 2000; Jung, 2012; Petereit et al., 2014).

Since Coombs (1981) introduced the density gradient column allowing for accurate measurements of egg specific gravity, a number of studies have been conducted focusing on i) the mechanisms of achieving egg buoyancy and parameterization of various egg components for egg specific gravity (Kjesbu et al., 1992; Jung et al., 2014), ii) the ecological significance of vertical egg distribution and variability in egg specific gravity for survival probabilities in relation to abiotic ambient conditions (e.g. Nissling et al., 1994; Ouellet, 1997; MacKenzie and Mariani, 2012), and iii) opportunities for retention in or dispersal to suitable habitats for development (e.g. Ospina-Álvarez et al., 2012; Myksvoll et al., 2013; Petereit et al., 2014). Hence, the vertical distribution of eggs as determined by egg specific gravity, and ambient salinity and temperature conditions, may have profound implications for the reproductive success and the year class-formation in teleosts.

The brackish water Baltic Sea, with restricted water exchange by shallow straights in the Sound and the Belt-Seas (ICES SD 22 and 23; Fig. 1), is characterized by a decrease in salinity in the surface water from ca 9 psu in the southwest (SD 24) to ca 3 psu in the north (SD 31), and a permanent halocline at 50–70 m depth with denser more saline, ca 10–20 psu, deep water in the Baltic proper (SD 24–28). Successful reproduction of marine fishes is restricted by opportunities for fertilization and egg development at low salinities (Nissling et al., 2002, 2006; Petereit et al., 2009) and/or by opportunities to obtain neutral egg buoyancy in the less dense water, i.e. not sink to the bottom or be subjected to unfavourable oxygen conditions in the deep layers (Nissling et al., 1994; MacKenzie et al., 2000). Conditions are strongly affected by highly irregular saline water inflow events, influencing both salinity and oxygen conditions and consequently the reproductive success and accordingly both abundance and distribution of marine fishes (Segerstråle, 1969; Ojaveer and Kalejs, 2005; MacKenzie et al., 2007), including flounder (Ojaveer et al., 1985; Drews, 1999; Ustups et al., 2013).

Flounder, *Platichthys flesus*, inhabit the Eastern Atlantic of Western Europe from the White Sea to the Mediterranean and the Black Sea including the Baltic Sea (Nielsen, 1986). In the Baltic Sea it display different spawning strategies; spawning along the coast and on offshore banks with demersal eggs in SD 26–30 and SD 32, and off-shore in the deep basins with pelagic eggs in SD 22–26 and SD 28 (Bagge, 1981), i.e. forming two genetically distinct (Hemmer-Hansen et al., 2007; Florin and Höglund, 2008) sympatric ecotypes, but sharing feeding and wintering areas (Nissling et al., 2015). Further, as an adaptation to the decrease in salinity; ca 15–25 psu in the Belt Seas and the Sound (SD 22 and 23), ca 13–20 psu in the Arkona Basin and Bornholm Basin (SD 24 and 25) vs ca 10–14 psu in the Gdansk Deep and Gotland Basin (SD 26 and 28) (Fig. 1), egg specific gravity of the ecotype with pelagic eggs has been shown to differ between spawning areas (Nissling et al., 2002; Petereit et al., 2014).

In the present study data of egg specific gravity from fish sampled in different Baltic Sea spawning areas in 2011–2015, together with already published data (Solemdal, 1971, 1973; Nissling et al., 2002; Petereit et al., 2014) were compiled with the aim to:

i) Explore the significance of egg diameter and egg dry weight respectively, for egg specific gravity, and hence for the opportunity to obtain neutral egg buoyancy along a salinity gradient, i.e. adaptation to prevailing water density.

ii) Reveal differences in egg characteristics between fish spawning in different areas to be used as a non-genetic tool in discrimination of subpopulations (Ciannelli et al., 2010; Myksvoll et al., 2013; Petereit et al., 2014).

iii) Assess the ecological significance of egg specific gravity and vertical egg distribution on egg survival probabilities of the flounder ecotype with pelagic eggs. Here we explore habitat suitability for egg survival at conditions prevailing before and after a major saline water inflow event into the Baltic Sea by comparing conditions in 2014 and in 2015 following the major inflow event in December–January 2014–2015 (Mohrholz et al., 2015) causing changes in salinity and thus in vertical egg distribution.

iv) Assess ontogenic changes in ESG until hatching of the ecotype with pelagic eggs as an increase in ESG during development (as shown for other species; see below) may imply development at less favourable oxygen concentrations.

In a broader context information about variability in egg specific gravity (and vertical egg distribution) of flounder may be used in stock-recruitment relationships by incorporation of hydrographic conditions and stock structure, i.e. estimate the effective spawning stock biomass (Hinrichsen et al., 2016b), and further, contribute to forecasting stock development in accordance with postulated deterioration of salinity and oxygen conditions in the deep basins as an effect of climate change affecting the extent of saline water inflows into the Baltic Sea (MacKenzie et al., 2007; Meier et al., 2012).

## 2. Materials and methods

### 2.1. Sampling of fish

The study includes measurements of egg characteristics of flounder, *Platichthys flesus*, sampled in ICES SDs 22–26 and SD 28 (Fig. 1) in 2011–2015. Sampling was conducted by either trawl surveys in the Baltic deep basins by R/V Alkor (SD 22–26 and SD 28) in April, or by gill net surveys from at 5 to at 70 m depth off eastern Gotland (SD 28) and in the Hanö Bight (SD 25) in mid April-early May (Fig. 1). Female size was measured as total length in mm or as cm length-class, the latter used in further analysis. Additionally, already published data (Solemdal, 1971, 1973; Nissling et al., 2002; Petereit et al., 2014) were included. In total 209 egg batches from different females were used. The sampling procedure is described in detail in Nissling et al. (2002) and in Petereit et al. (2014) (Raw data available at the following link: https://doi.pangaea.de/10.1594/PANGAEA.871590).

### 2.2. Sampling of eggs

Eggs for measurements were obtained by stripping and artificial fertilization, using eggs from one female and semen from 2 to 3 males, either directly on board (R/V Alkor) or after transportation to the Ar Research Station or to a mobile laboratory where fish

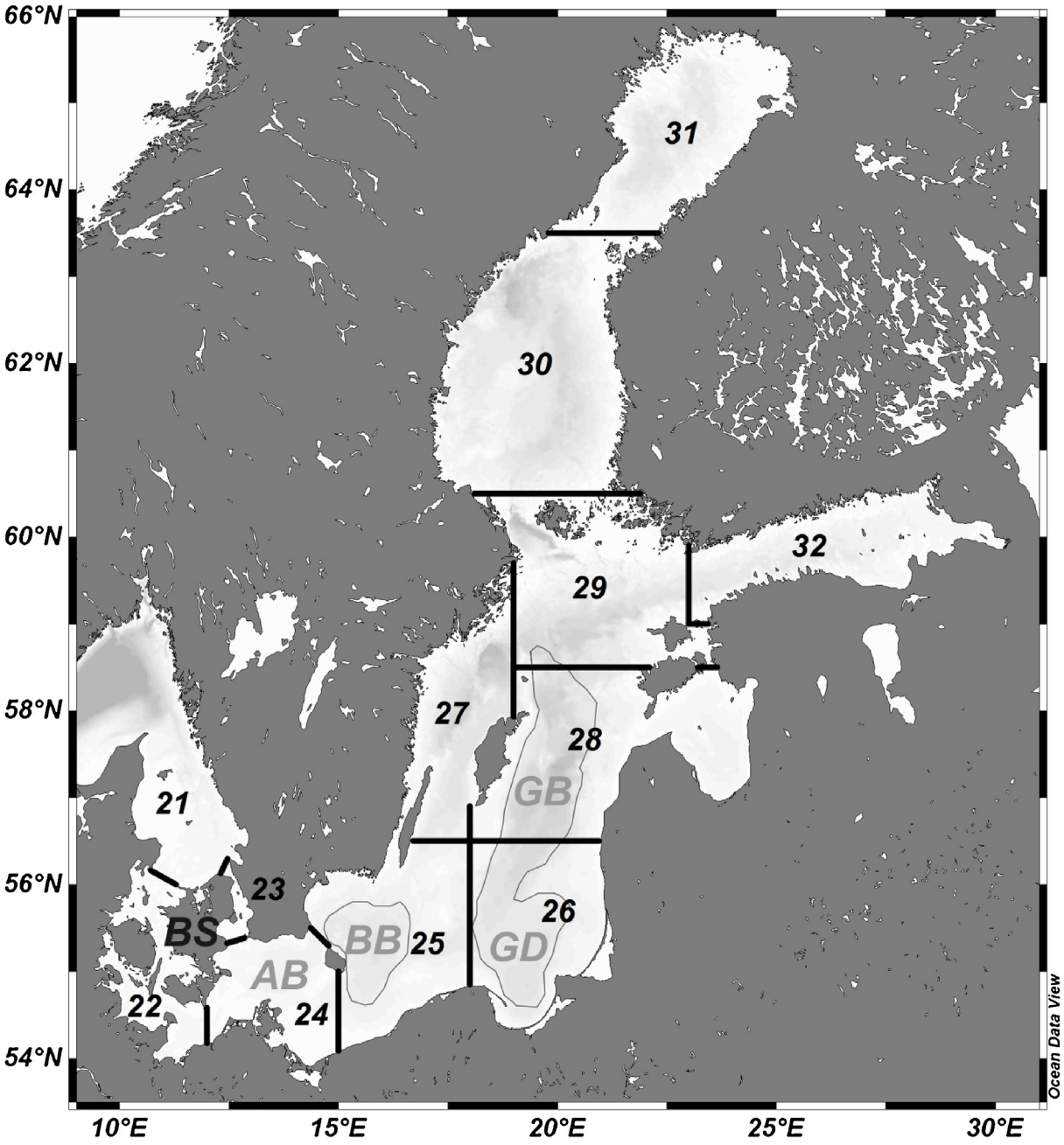


**Fig. 1.** ICES subdivisions (SD) in the Baltic Sea with spawning areas of flounder, *Platichthys flesus*, with pelagic eggs: BS – Belt Sea (SD 22), AB – Arkona Basin (SD 24), BB – Bornholm Basin (SD 25), GD – Gdansk Deep (SD 26), GB – Gotland Basin (SD 28).

were kept in tanks with running water until stripping. Fertilization was performed at 17–20 psu at ca 6–10 °C. After 0.5–1 h a subsample of fertilized eggs were incubated in new water at 6–9 °C and 17–20 psu until measurements ca 12–24 h post fertilization. Eggs used for measurements of specific gravity, diameter and dry weight were scanned under a stereo-microscope and only eggs with normal development (i.e. regular cell morphology; Kjørsvik et al., 1990) were used to ensure only high quality eggs in measurements.

### 2.3. Determination of egg characteristics

Egg specific gravity (ESG) was determined using a density gradient column (Coombs, 1981) using 15–30 eggs in stage IA (Thompson and Riley, 1981) in each determination. The eggs were inserted in the column and, after a settling time of ca 45–60 min, the positions recorded and compared with the positions of 4–9 density glass floats (Spartel, UK; Martin Instruments Co, UK) of known specific gravity; correlation coefficient of the density floats were >0.99 in all measurements. Measurement were performed at ca 7 °C; if deviating from this (temperature checked at the top of the water column) ESG was adjusted to 7 °C using a seawater density calculator (see link below). The procedure for determination of ESG is described in detail in Nissling et al. (2002) and in Petereit et al. (2014).

In nine egg batches from different females, eggs were incubated in the density gradient column throughout development from stage IA (12–24 h after fertilization) until hatching, i.e. at neutral buoyancy. ESG was assessed 1–2 times per day to record changes during ontogeny; development stage, according to Thompson and Riley (1981), determined using egg batches from the respective female

incubated at similar conditions (at 20 psu and 6–8 °C). Incubation of a limited number of eggs in a density gradient column implies no effect on egg development, resulting in 82–100% viable larvae; see Petereit et al. (2014) for more details.

Egg diameter, assessed as the outside diameter set by the chorion, was measured by one cross diagonal measurement at stage IA under a stereo-microscope at 50 or at 240 x magnification using an ocular micrometre scale, with 20–30 eggs in each determination. Egg dry weight (stage IA) was assessed in two ways, either 6–8 eggs, rinsed in demineralized water 2 times for ca 10 s, were collected in a pre-weighted aluminium vial for freeze drying (at −50 °C) and subsequently weighted to the nearest 0.1 μg (Sartorius microbalance SC2), or batches of 200 eggs, rinsed 2 times for ca 15 s in demineralized water, was incubated at 60 °C for 24 h and weighted (0.1 mg; Sartorius BP210S).

### 2.4. Hydrographic data and vertical egg distribution

Salinity, temperature and oxygen data were measured using a CTD provided with an oxygen probe (ADM) with continuous measurements from the surface to 3 m above the bottom in the main spawning areas for the flounder ecotype with pelagic eggs (see Hinrichsen et al., 2016a), the Bornholm Basin (SD 25), the Gdansk Deep (SD 26) and the Gotland Basin (SD 28). The measurements were carried out during surveys with R/V Alkor in April 2014 and in April 2015; data from station BB25, GD60 and GB90 (cruise Reports Al435 (DOI 10.3289/CR_AL435] and Al454 [http://oceanrep.geomar.de/id/eprint/28939]).

By combining data of water density from CTD casts with mean ESG of each egg batch, the vertical egg distribution was determined in accordance to the neutral egg buoyancy. As no difference in ESG was found between eggs from SD 24 and SD 25, and between eggs from SD 26 and SD 28, respectively, the vertical egg distribution in the Bornholm Basin (SD 25) was assessed using egg batches from SD 24 and SD 25 pooled (n = 42), and the vertical egg distribution in the Gdansk Deep (SD 26) and the Gotland Basin (SD 28) with egg batches from SD 26 and SD 28 pooled (n = 69); see below.

### 2.5. Habitat suitability

Habitat suitability for egg survival (Hinrichsen et al., 2016c) was assessed as the water column at which eggs would obtain neutral buoyancy above critical levels for development, i.e. 1 ml oxygen $l^{-1}$ (Vitinsh, 1980; Grauman, 1981) and >2 °C (Hinrichsen et al., 2016b; Isa Wallin, personal communication). Accordingly, egg survival probabilities and cause of mortality were evaluated in relation to temperature and oxygen conditions at the depth where the respective egg batch achieve neutral buoyancy (at stage IA), or as death from sedimentation of non-buoyant egg batches; no eggs of the ecotype with pelagic eggs are expected to survive conditions prevailing at the bottom (see Hinrichsen et al., 2016c; and references therein).

## 3. Results

### 3.1. Differences in egg specific gravity between areas

Egg specific gravity (ESG) at stage IA (Thompson and Riley, 1981), measured at 7 °C ca 12–24 h post fertilization, varied between areas. The highest values occurred in SD 23, 1.02038 ± 0.00062 (mean ± sd) g $cm^{-3}$ and the lowest in SD 26 and SD 28, 1.00955 ± 0.00074 g $cm^{-3}$ and 1.00955 ± 0.00068 g $cm^{-3}$, respectively (Table 1; Fig. 2). A GLM (general linear model; SPSS ver. 22) with ESG as dependent variable, area (ICES SD) as fixed factor and female length-class as covariate resulted in a significant effect of area, df = 5, F = 16.58, p < 0.001 (data from SD 23 and for demersal eggs in SD 25 were excluded in analysis due to few measurements). Pairwise comparisons revealed three groups for flounder with pelagic eggs, SD 22, SD 24 and 25, and SD 26 and 28, respectively separated from flounder with demersal eggs in SD 28 (Table 2). A weak positive effect (df = 1, F = 4.18, p = 0.042) of length-class on ESG occurred. This effect was however related to measurements in SD 22 only. Excluding SD 22 in analysis resulted in no effect (df = 1, F = 0.410, p = 0.523) with p = 0.245-0.645 for the respective group, SD 24 and 25, SD 26 and 28 and SD 28 with demersal eggs, i.e. no evident effect of female size on ESG. Apart from that measurements in SD 22, in contrast to other areas, yielded a positive relationship between length-class and ESG, variability in ESG was high, standard deviation of 0.0021 g $cm^{-3}$ compared to 0.0007-0.0013 g $cm^{-3}$ for the other areas (Table 1). The results from SD 22 may be related to sampling occasion (trawl station and/or date) potentially and effect of mixed populations in the area (Petereit et al., 2014; discussion below).

### 3.2. Significance of egg diameter and egg dry weight

Regression analysis revealed a strong effect of egg diameter on ESG, df = 1, F = 582.1, p < 0.001, but no effect of egg dry weight on ESG, df = 1, F = 0.00, p = 0.98 (Fig. 3a and 3b) suggesting that variability in ESG among subpopulations/areas is related mainly to differences in water content, manifested as differences in egg diameter, but with similar dry weights among areas (Table 1). This was supported by an ANOVA analysis with egg diameter as dependent variable and SD as fixed factor, resulting in a significant difference between SDs, df = 4, F = 63.57, p < 0.001. A corresponding ANOVA with egg dry weight as dependent variable yielded a significant difference between areas, df = 4, F = 3.19, p = 0.017. However, this was due to a significant difference between SD 25 (N.B. n = 9) and SD 26 (p = 0.015; pairwise comparisons) whereas no differences occurred between e.g. eggs from SD 22 and pelagic eggs in SD 28 (p = 1.00) or between pelagic and demersal eggs in SD 28 (p = 1.00). Hence, variability in ESG among subpopulations/areas is related to differences in egg diameter (i.e. water content) with no difference in egg dry weight.

A multiple regression model suggested inclusion of both egg diameter (df = 1, t = 25.53, p < 0.001; negative relationship) and egg dry weight (df = 1, t = 8.15, p < 0.001; positive relationship) explaining 87% of the variation in ESG (df = 2, F = 326.0, p < 0.001) when measurements from all SDs were pooled, i.e. larger eggs with lower dry weight result in more buoyant eggs. This was valid also for SD 22 and SD 26 and 28, i.e. with pelagic eggs, and close to significant for SD 28 with demersal eggs (Table 3), i.e. within the respective sub-population/area variability in ESG is determined by both egg diameter (i.e. water content) and dry weight.

### 3.3. Ontogenic changes in egg specific gravity

ESG during development in nine egg batches from early stage 1A to stage IV and hatching is presented in Fig. 4. In general, ESG decreased somewhat during development (Fig. 4a and c), or remained more or less stable (Fig. 4b) until mid/late stage III followed by an increase in ESG during stage IV until hatching. A decrease in ESG during development result in an upward movement of the egg (until stage III) followed by sinking during stage IV until hatching. The highest decrease in ESG up to stage III was approximately 0.0015–0.0020 g $cm^{-3}$ corresponding, roughly, to ca 50% of the variability in ESG of egg batches in stage early IA within the respective area (based on the variability between egg batches in SD 24 and 25, and SD 26 and 28, respectively) and may thus increase opportunities for egg survival as evaluated below. Similarly, the highest increase in ESG during stage IV, ca 0.0015 g $cm^{-3}$ compared

**Table 1**
Egg characteristics of flounder, *Platichthys flesus*, in different ICES subdivisions (SD) in the Baltic Sea (mean ± sd) together with female size of analyzed fish. In SD 25 and SD 28, where both ecotypes occur, P indicates pelagic eggs and D demersal eggs. Number of measurements (egg batches from different females) within brackets.

| SD | Egg specific gravity (g cm$^{-3}$) | Egg diameter (mm) | Egg dry weight (μg) | Female size (cm) |
|---|---|---|---|---|
| SD 22 | 1.01520 ± 0.00206 (33) | 1.08 ± 0.06 (33) | 37.9 ± 5.0 (32) | 35.5 ± 6.3 |
| SD 23 | 1.02038 ± 0.00062 (4) | | | 30.0 ± 4.1 |
| SD 24 | 1.01220 ± 0.00122 (13) | | | 30.8 ± 2.8 |
| SD 25P | 1.01129 ± 0.00123 (29) | 1.17 ± 0.07 (18) | 34.5 ± 2.5 (9) | 31.3 ± 4.4 |
| SD 25D | 1.01663 ± 0.00057 (3) | 1.06 (1) | | 30.0 ± 3.8 |
| SD 26 | 1.00955 ± 0.00074 (17) | 1.26 ± 0.06 (17) | 41.1 ± 5.9 (16) | 32.2 ± 5.6 |
| SD 28P | 1.00955 ± 0.00068 (52) | 1.26 ± 0.05 (36) | 37.2 ± 3.9 (33) | 30.0 ± 3.8 |
| SD 28D | 1.01601 ± 0.00076 (58) | 1.02 ± 0.04 (35) | 36.5 ± 6.5 (11) | 29.7 ± 4.9 |

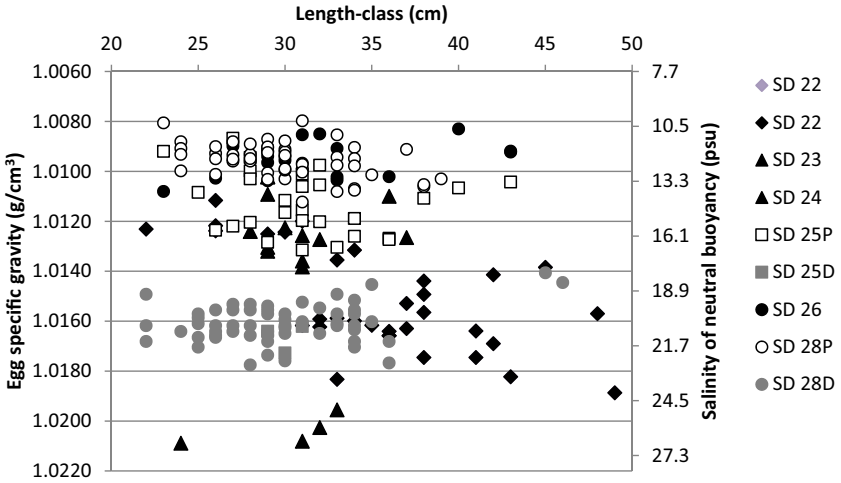


**Fig. 2.** The relationship between egg specific gravity (g cm$^{-3}$) and female size (cm length class) for flounder, *Platichthys flesus*, in different ICES subdivisions (SD). Corresponding salinity of neutral buoyancy at 7 °C shown on second vertical axis.

**Table 2**
Pairwise comparisons of egg specific gravity of flounder, *Platichthys flesus*, in different ICES subdivisions (SD) by GLM-analysis (evaluated at length class 31.25 cm). In SD 25 and SD 28 where both ecotypes occur P indicates pelagic eggs and D demersal eggs.

| SD | mean | 95% lower | 95% upper | SD 22 | SD 24 | SD 25P | SD 26 | SD 28P | SD 28D |
|---|---|---|---|---|---|---|---|---|---|
| SD 22 | 1.0143 | 1.01389 | 1.01474 | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| SD 24 | 1.0122 | 1.01165 | 1.01278 | <0.001 | | 0.100 | <0.001 | <0.001 | <0.001 |
| SD25P | 1.0113 | 1.01091 | 1.01166 | <0.001 | 0.100 | | <0.001 | <0.001 | <0.001 |
| SD 26 | 1.0096 | 1.00911 | 1.01010 | <0.001 | <0.001 | <0.001 | | 1.000 | <0.001 |
| SD 28P | 1.0096 | 1.00935 | 1.00994 | <0.001 | <0.001 | <0.001 | 1.000 | | <0.001 |
| SD 28D | 1.0160 | 1.01573 | 1.01628 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | |

**Table 3**
Results from multiple-regression analysis between egg specific gravity (dependent variable) and egg diameter and egg dry weight, for flounder, *Platichthys flesus*, in different ICES subdivisions (SD); for all areas pooled and for the respective sub-population. P = pelagic eggs. D = demersal eggs. n = number of egg batches.

| | Egg diameter | | Egg dry weight | | | Model summary | | |
|---|---|---|---|---|---|---|---|---|
| | t | p | t | p | n | F | adjusted r$^2$ | p |
| SDs pooled | −25.5 | <0.001 | 8.15 | <0.001 | 100 | 326.0 | 0.868 | <0.001 |
| SD 22 | −16.49 | <0.001 | 12.53 | <0.001 | 32 | 151.6 | 0.907 | <0.001 |
| SD 25P | −1.56 | 0.181 | −0.218 | 0.836 | 8 | 1.90 | 0.205 | 0.243 |
| SD 26 and 28P | −5.24 | <0.001 | 3.21 | <0.01 | 49 | 15.30 | 0.373 | <0.001 |
| SD 28D | −2.65 | <0.05 | 1.81 | 0.108 | 11 | 3.61 | 0.343 | 0.077 |

to the initial ESG, involve sinking and potentially increased egg mortality.

### 3.4. Egg survival probabilities

In Fig. 5 vertical profiles of temperature, salinity and oxygen concentration in April 2014 and in April 2015, are shown for the Baltic Sea deep basins, the Bornholm Basin (SD 25), the Gdansk Deep (SD 26) and the Gotland Basin (SD 28). The major saline water inflow event in the winter 2014–2015 resulted in improved conditions for egg development below the halocline, i.e. in the main spawning areas for flounder with pelagic eggs. In the Bornholm Basin and the Gdansk deep both salinity and oxygen concentrations increased whereas in the Gotland basin there was an increase in salinity while oxygen conditions remained low. The on average salinity below the halocline increased from 13.7 ± 2.0 (mean ± sd) psu in April 2014 to 15.5 ± 2.6 psu in April 2015 in the Bornholm Basin, from 10.6 ± 1.3 psu to 13.8 ± 1.6 psu in the Gdansk Deep, and from 9.4 ± 1.0 psu to 11.0 ± 1.3 psu in the Gotland Basin. Higher salinities (denser water) resulted in a significant change in depth range

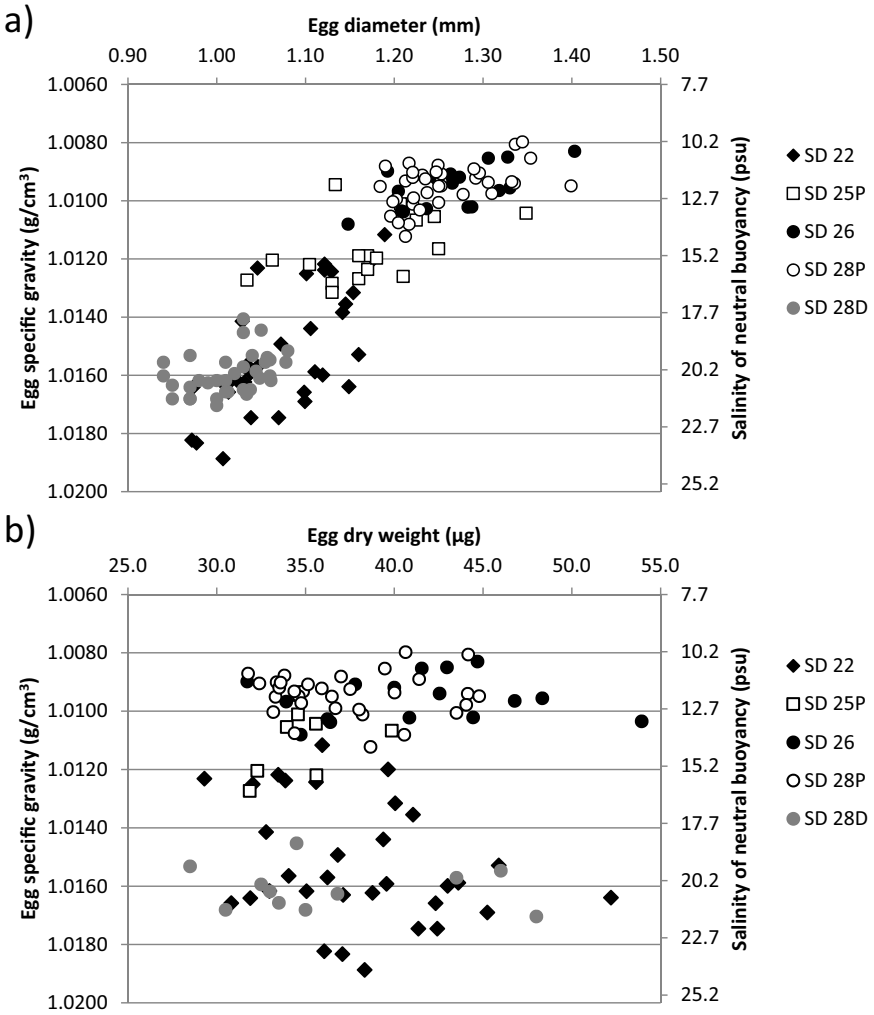


**Fig. 3.** The relationship between egg specific gravity (g cm$^{-3}$) and a) egg diameter (mm) and b) egg dry weight (µg) for flounder, *Platichthys flesus*, in different ICES subdivisions (SD). Corresponding salinity of neutral buoyancy at 7 °C shown on second vertical axis.

**Table 4**
Average depth (m) and depth range for studied flounder, *Platichthys flesus*, egg batches obtaining neutral buoyancy within the water column in 2014 and 2015, and the average temperature (°C), salinity (psu) and oxygen concentration (ml l$^{-1}$) experienced by these egg batches in ICES subdivisions (SD) 25, 26 and 28 respectively. Mean ± standard deviation. n refers to the number of egg batches obtaining neutral buoyancy in the water column, with the total number of tested batches within brackets.

| | SD 25 | | | | SD 26 | | | | SD 28 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2014 | 2015 | t | p | 2014 | 2015 | t | p | 2014 | 2015 | t | p |
| Depth | 61.4 ± 6.9 | 58.1 ± 4.3 | 2.31 | <0.05 | 91.8 ± 3.3 | 83.2 ± 2.9 | 14.58 | <0.001 | 108.6 ± 9.6 | 94.6 ± 10.6 | 3.13 | <0.001 |
| Depth range | 51–72 | 52–69 | | | 80–98 | 79–91 | | | 94–118 | 75–110 | | |
| Temperature | 6.5 ± 1.0 | 7.4 ± 0.5 | | | 5.9 ± 0.4 | 6.5 ± 0.5 | | | 5.1 ± 0.2 | 5.8 ± 0.3 | | |
| Salinity | 14.2 ± 1.4 | 15.1 ± 1.5 | | | 11.7 ± 0.6 | 12.2 ± 0.8 | | | 10.6 ± 0.4 | 11.1 ± 0.5 | | |
| Oxygen | 3.3 ± 1.4 | 4.0 ± 0.9 | 2.20 | <0.05 | 1.3 ± 0.8 | 2.8 ± 0.6 | 10.94 | <0.001 | 0.3 ± 0.2 | 0.5 ± 0.3 | 2.33 | <0.05 |
| Batches (n) | 31 (42) | 42 (42) | | | 46 (69) | 68 (69) | | | 7 (69) | 24 (69) | | |

at which eggs obtained neutral buoyancy (Fig. 5), from on average 61.4 m to 58.1 m in the Bornholm Basin, 91.8 m to 83.2 m in the Gdansk deep, and 108.6 m to 94.6 m in the Gotland Basin, and thus at more favourable oxygen conditions (Table 4).

The change (uplift) in depth range for egg development between 2014 and 2015 affected egg survival probabilities significantly. In the Bornholm Basin and the Gdansk Deep opportunities for egg survival increased from 26% to 100% ($p < 0.001$; Fisherís exact test) and from 32% to 99% ($p < 0.001$) respectively, whereas probabilities for egg survival in the Gotland Basin was low in both years, 0% and 3% ($p = 0.496$), respectively (Fig. 6). The habitat suitability was improved due to both more favourable oxygen conditions and to increased opportunities for remaining neutrally buoyant within the water column (Fig. 6; Table 4). Temperature had no impact on egg survival probabilities as temperatures were within preferred preferences for egg development (4–10 °C; Hinrichsen et al., 2016b; Isa Wallin, personal communication) in all basins in both years.

## 4. Discussion

In principle, egg specific gravity of fish eggs is determined by the fractional contributions of the main components, yolk + embryo, chorion and the perivitelline space between the chorion and vitelline membrane (Craik and Harvey, 1987; Kjesbu et al., 1992;

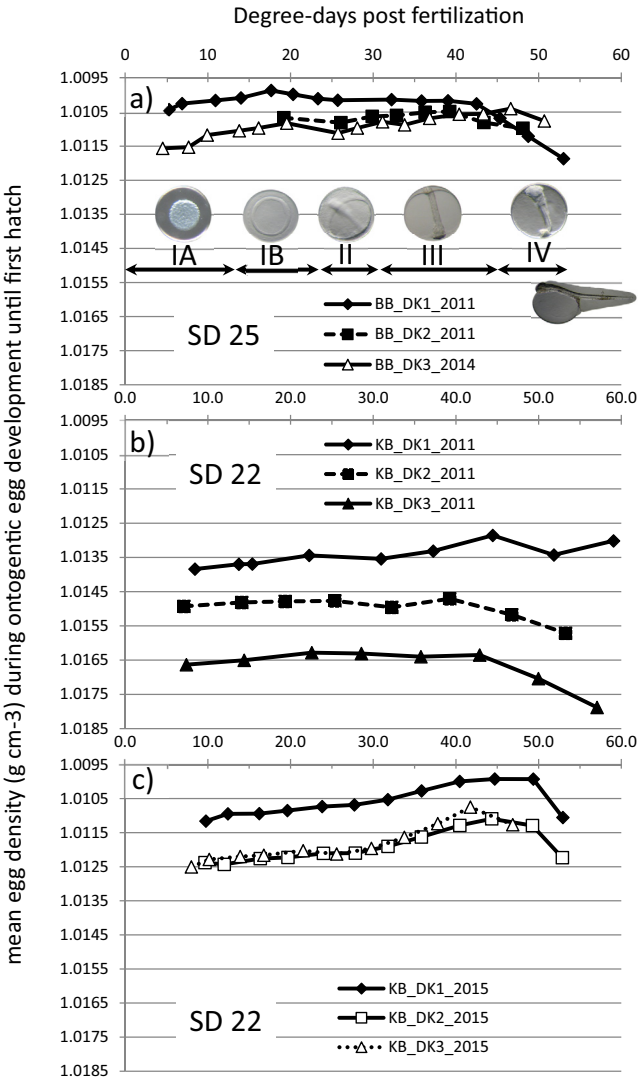


**Fig. 4.** Mean values of egg specific gravity of flounder, *Platichthys flesus*, during ontogenesis from stage IA to stage IV and hatching from two ICES subdivisions (SD), 25 (a) and 22 (b and c). Data in Panel B are taken from Petereit et al. (2014) and are from February/March. Data shown in Panel A and C are from experiments in April.

Govoni and Forward, 2008; Sundby and Kristiansen, 2015) with the high water content of the yolk being a key factor compensating for the heavy components, proteins, in the chorion and embryo; see e.g. Jung et al. (2014) or Sundby and Kristiansen (2015) for parameterization of the respective component for the overall ESG. In addition to the high water content of the yolk, also oil droplets and yolk lipids, with lower density than seawater, may contribute to egg buoyancy in some species (Cerdà et al., 2007; Govoni and Forward, 2008).

For flounder, *Platichthys flesus*, in the Baltic Sea, ESG at early stage IA varied significantly between areas in accordance with the decrease in salinity, i.e. the highest values ($1.0152 \pm 0.0021$ and $1.0204 \pm 0.0006\ g\ cm^{-3}$; mean ± sd) were obtained in the western parts, SD 22 and SD 23, and the lowest ($1.0096 \pm 0.0007\ g\ cm^{-3}$) in the eastern, SD 26 ad SD 28, i.e. allowing eggs to obtain neutral buoyancy in the respective spawning area. ESG of flounder spawning demersal eggs, sampled in SD 25 and SD 28, is an exception with ESG of $1.0161 \pm 0.0008\ g\ cm^{-3}$, a strategy adopted as spawning at low salinities (ca 6–8 psu) prevents eggs to achieve buoyancy. Similar values of ESG, 1.0151–1.0157 g cm$^{-3}$, for flounder spawning demersal eggs have been obtain in SD 27 and SD 29/32 (Solemdal, 1971, 1973). Hence, obtained results suggest that the ecotype with pelagic eggs may be separated into three subpopulations (SD 22, SD 24 and 25, and SD26 and 28, respectively), with different egg characteristics (ESG) in accordance with salinity conditions in the respective spawning area, separated from the ecotype with demersal eggs. N.B. data from SD 23 were excluded in comparison due to few measurements. In SD 22 ESG varied between 1.01116 and 1.01887 g cm$^{-3}$ corresponding to a salinity of ca 14.3–24.2 psu, i.e. the variability in ESG was substantial. Salinity in the area varies between ca 14 and 25 psu but according to data in Petereit et al. (2014) about 60% of flounder egg batches had a higher ESG compared to the water density (measured by CTD-casts) in 3 out of 6 sampling areas. Similar results were obtained by von Westernhagen et al. (1988), 38 of 60 egg batches (65%) did not correspond to the ambient salinity. The areas SD 22 and SD 23 are mixing areas between the Baltic and the Kattegat, with potentially more than one population utilizing the area as discussed by Petereit et al. (2014), potentially causing the high variability in ESG. Obtained ESG for flounder in the Baltic Sea differ significantly from ESG of flounder at marine conditions, ca 1.0246 g cm$^{-3}$ (Solemdal, 1971). As evident from reciprocal experiments, egg characteristics of marine and brackish water eggs remain essentially unchanged when fish are transferred from brackish to a marine environment and vice versa; Solemdal (1971, 1973) for flounder and Nissling and Westin (1997) for cod. Hence, with respect to egg characteristics fish in the Baltic Sea form specific populations. A similar adaptive pattern is also assumed for species spawning in the brackish water Black Sea, specifically blue fin tuna, *Thunnus thynnus* (MacKenzie and Mariani, 2012).

Although multiple regression analysis showed that ESG in Baltic flounder was related to both egg diameter (i.e. water content; Thorsen et al., 1996) and egg dry weight (mass of mainly proteins) within the respective subpopulation/area, differences in ESG between areas/subpopulations were related only to egg diameter, with larger eggs in the eastern parts of the Baltic Sea than in the western, while egg dry weight was the same irrespective of area/subpopulation, including demersal eggs. This indicates that water content acquired in the ovary during oocyte hydration by a breakdown of yolk proteins to FAA as osmotic effectors and a corresponding inflow of water (Thorsen et al., 1996; Finn and Fyhn, 2010) mediated by a selective influx of water by channel membrane proteins, aquaporins (Fabra et al., 2005; Cerdà, 2009) is the key mechanism. This process is considered highly regulated and potentially species specific (Cerdà, 2009) and, as shown by Thorsen et al. (1996), differences in the degree of breakdown of yolk protein and thus in the osmotic potential, result in a higher inflow of water during hydrolysis in cod eggs at brackish water conditions compared to at marine conditions. Concomitant to this, the results suggest that the same amount of material is deposited in the egg irrespective of area and spawning strategy, and that differences in ESG between subpopulations are determined by differences in the water content, potentially due to differences in the degree of degradation of yolk proteins into FAA during hydrolysis.

Within the respective area/subpopulation however, both variability in egg diameter and in egg dry weight determine ESG, and result in differences in vertical egg distribution with implications for egg survival probabilities. This is in agreement with studies on other species, e.g. for cod, intra-population variability in ESG is caused by differences in both the amount of material deposited into the chorion, and to the degree of uptake of water during hydration prior to ovulation; i.e. to the water content/egg diameter (Kjesbu et al., 1992; Jung et al., 2014).

Incubation of eggs from early stage IA to stage IV showed that ESG may change during ontogeny up to hatching; a slight decrease in ESG during development was noted for egg batches sampled in SD 22 and SD 25 in April, with an initial ESG of

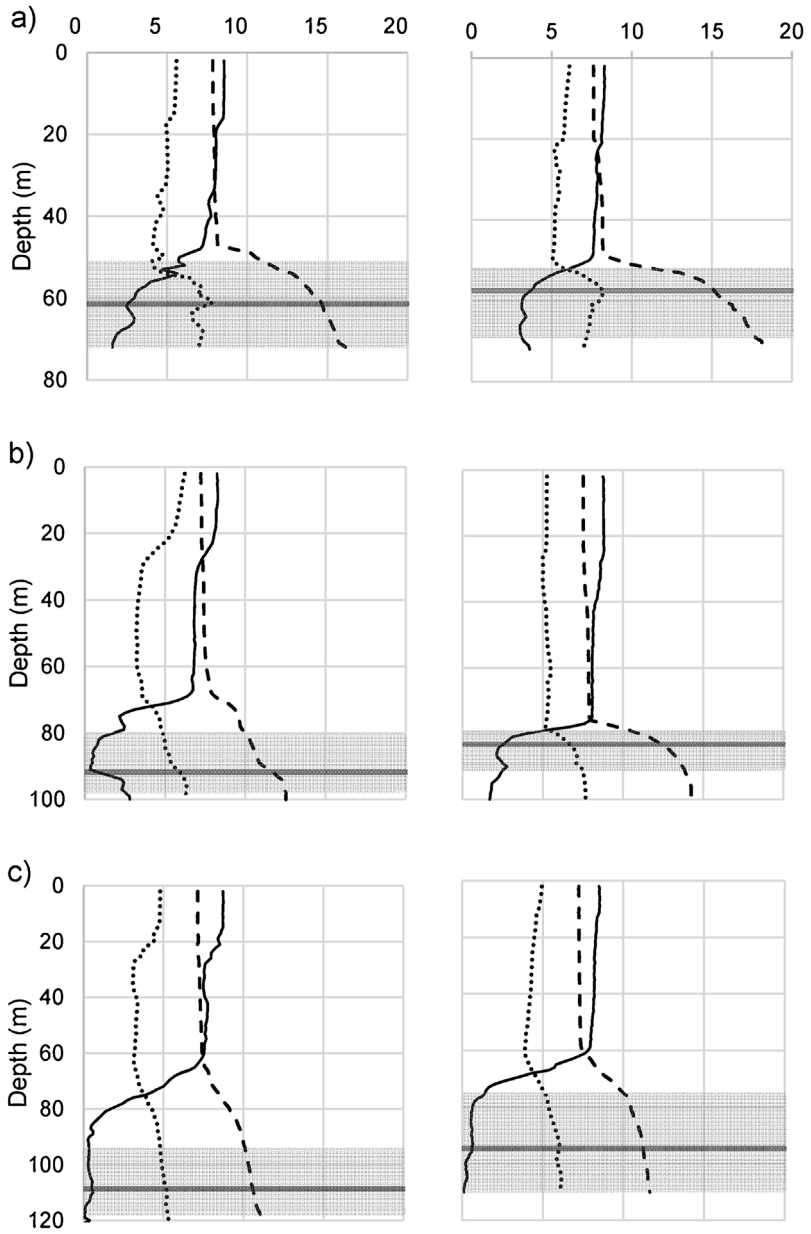


**Fig. 5.** Depth profiles showing temperature (°C; dotted line), oxygen (ml $l^{-1}$; solid line) and salinity (psu; dashed line) in ICES subdivisions a) 25, b) 26 and c) 28 (Fig. 1) in April 2014 (to the left) and April 2015 (to the right), and depth range (grey horizontal field) and mean depth (horizontal line) of flounder, *Platichthys flesus*, egg batches that would obtain neutral buoyancy (see Table 4); based on egg specific gravity and hydrographic data from CTD-casts in the respective area.

1.0104–1.0125 g $cm^{-3}$, whereas ESG for egg batches from SD 22 sampled in February–March, with an initial ESG of 1.0138-1.0166 g $cm^{-3}$, remained stable until stage IV. The results contrast to the general picture of changes in ESG during ontogeny of marine fishes (Jung et al., 2014), i.e. a slight increase in ESG until mid-gastrulation (stage II), followed by a gradual decrease in ESG, and finally an increase in ESG just prior to hatching. As concluded by Jung et al. (2014), changes in ESG during development are associated with changes in the yolk + embryo fraction with a corresponding change in the perivitelline space. As opposed to the chorion which is permeable to water, the yolk membrane is impermeable to water following the cortical reaction at fertilization but with an increasing permeability during egg development (Mangor-Jensen, 1987), i.e. density of the pervitelline space is in equilibrium with the surrounding environment whereas density of the yolk + embryo changes during ontogeny. Prior to the gastrulation there is a decrease in the volume of yolk + embryo (i.e. water content) and a corresponding increase in volume of the pervitelline space, which is reversed following gastrulation as osmoregulation organs start to function (Riis-Vestergaard, 1987). Apart from differences between species in size of the pervitelline space (e.g. in sardine, *Sardina pilchardus*, the pervitelline space occupy >80% of the egg volume, and ca 74% in European eel, *Anguilla anguilla*, compared to <10% for the majority of pelagic fish eggs; Coombs et al., 2004; Sørensen et al., 2015), ambient salinity conditions can be expected to involve differences in the relation between volume of yolk + embryo and volume of the pervitelline space. For Baltic cod eggs incubated at different salinities, ESG decreased during development in batches incubated at 10 psu but increased when incubated at 17 psu and in particular at 30 psu (Nissling and Vallin, 1996). Thus, changes in ESG during development may be related to initial ESG. Being neutrally buoyant at low salinities may involve lower loss of water initially as the difference between external and internal osmolality is low; internal osmolality correspond-

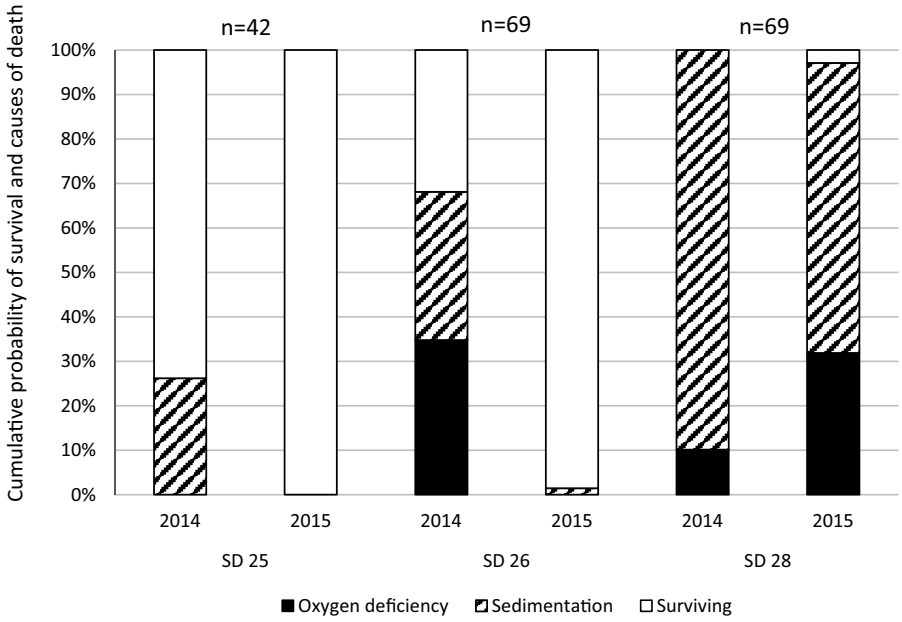


**Fig. 6.** Assessed cumulative causes of mortality (due to oxygen deficiency or sedimentation) and survival probabilities of flounder, *Platichthys flesus*, eggs in ICES subdivisions (SD) 25, 26 and 28 (Fig. 1) in April 2014 and April 2015, respectively. n refer to number of egg batches.

ing to a salinity of ca 11 psu (Sundby and Kristiansen, 2015), i.e. only a minor decrease in the volume of yolk + embryo. Moreover, low ambient salinity may facilitate water uptake after gastrulation, i.e. an increase in the volume of yolk + embryo, influencing ESG. Furthermore, low ambient salinity result in lower density of the perivitelline space compared to at marine conditions (as the chorion is permeable to seawater, density of the perivitelline space is equal to the density of ambient water) influencing the overall ESG. Despite that the contribution of the respective egg component was not studied, results of the present study suggest that ambient salinity may be of significance for changes in ESG during ontogeny; development at low salinities, ca 13.5–16 psu (Fig. 4a and c) involved a decrease in ESG, and in egg batches with an initial ESG, corresponding to 17.7–21.3 psu (Fig. 4b), ESG remained more or less stable until stage IV (in contrast to what is known for studied species at marine conditions; Jung et al., 2014).

Major saline water inflows into the Baltic Sea result in improved salinity and oxygen conditions (Matthäus and Franck, 1992; Matthäus and Lass, 1995) and thus increased habitat suitability (increased opportunities to keep buoyant in the water column and decreased risk of being subjected to lethal oxygen conditions) for fish with pelagic eggs, as shown in earlier studies for both cod (Nissling et al., 1994; MacKenzie et al., 2000; Köster et al., 2005) and flatfishes (Nissling et al., 2002; Ustups et al., 2013), a prerequisite for the formation of strong year classes. In general, the most favourable conditions occur in the Bornholm Basin compared to in the Gdansk Deep and Gotland Basin given the relative distance from the shallow sills in SD 22 and SD 23. The Baltic Sea is heavily affected by eutrophication and, as a consequence, large parts of the deep areas are oxygen depleted with regularly negative oxygen values (Conley et al., 2002; Diaz and Rosenberg, 2008). This implies that the effect of inflow events on habitat suitability for fish reproduction nowadays is limited and transitory, with soon deteriorated conditions (see MacKenzie et al., 2007). The situation is further aggravated by less frequent inflow events during the last decades, potentially an effect of climate change (Meier et al., 2012). The inflow event in 2014 improved habitat suitability for flounder eggs significantly in the Bornholm Basin (SD 25) and the Gdansk Deep (SD 26) but was not potent to significantly improve conditions in the Gotland Basin (SD 28).

Highly fluctuating conditions for egg survival of flounder (Hinrichsen et al., 2016b; present study) may affect both stock abundance and distribution, although stock development additionally is influenced by e.g. retention/dispersal of early stages (Petereit et al., 2014 Hinrichsen et al., 2016a), conditions in the nursery areas (e.g. Iles and Beverton, 1998, 2000), as well as fishing mortality. Poorer conditions for egg survival in the Gotland Basin at present are in accordance with the current stock development (see Hinrichsen et al., 2016b); decreasing CPUE in SD 28 but stable CPUE in SD 25 and SD 26. Evaluation of stock development is however hampered by the occurrence of the two flounder ecotypes with different spawning strategies and requirements for egg survival (Nissling et al., 2002) and thus recruitment. According to ICES (2015) the ecotype with pelagic eggs consist of three different subpopulations occurring in SD 22–23, SD 24–25 and in SD 26 and SD 28, respectively, whereas the ecotype with demersal eggs is considered as one stock, present in SD 27 and in SD 29–32. With respect to egg characteristics the present study confirms the suggestion of two populations of flounder with pelagic eggs in the Baltic Proper (one in SD 24 and SD 25, and another in SD 26 and SD 28) separated from flounder in the Belt-Seas (SD 22). Concerning the ecotype with demersal eggs, however the present study shows, in contrast to as suggested (ICES, 2015) that this type of flounder occurs in both SD 25 and SD 28. That the ecotype with demersal eggs inhabit not only the northern parts of the Baltic Proper; e.g. ESG of ca 1.0151–1.0157 g $cm^{-3}$ in SD 27 and SD 29/32 (Solemdal, 1971, 1973), i.e. with ESG equal to the results in present investigation (suggesting one homogenous population), but occur also in the southern Baltic is consistent with earlier observations; demersal flounder eggs have been found at the Oder Bank and at the Adlergrund in SD 24 (e.g. Mielck and Künne, 1932). The stock abundance of the ecotype with demersal eggs in the southern parts is, however, poorly known. Anyway, the suggested stock structure (ICES, 2015) should be revised to disentangle stock development of the respective flounder ecotype and for evaluation of stock-recruitment relationships of subpopulations. Further, presented data of egg specific gravity, and thus in vertical egg distribution and egg survival, may be used to estimate variability in the viable egg production in different areas/subpopulations, i.e. contribute to forecast stock development of Baltic Sea flounder at potentially

deteriorating salinity and oxygen conditions and higher temperatures following the effect of climate change (MacKenzie et al., 2007; Meier et al., 2012). E.g. based on salinity and oxygen requirements for egg survival (Hinrichsen et al., 2016b) including opportunities to obtain neutral egg buoyancy (present study), it can be expected that i) the distribution of flounder in the Baltic Sea will be compressed to the southern parts due to insufficient salinity for fertilization and egg development affecting recruitment in the northern parts (Nissling et al., 2002) and due to poorer habitat suitability in the deep basins, in particular the Gotland Basin (present study), and ii) that the relative contribution of the ecotype with demersal eggs spawning in coastal areas may increase as the reproductive success of this spawning strategy is less dependent on major saline water inflows events.

## Ethical approval

All applicable international, national, and institutional guidelines for the care and use of animals were followed.

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